INTEGRATED SYSTEMS

Nutritive Value and Animal Selection of Forage Chicory Cultivars Grown in Central Appalachia

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ABSTRACT

The unequivocal success of 'Grasslands Puna' (Puna) chicory (*Cichorium intybus* L.) as a forage species in other areas has not been realized in the central Appalachian Region of the USA. A field study was conducted in southern West Virginia (38° N, 81° W; 850 m above sea level) to compare nutritional qualities and palatability of herbage from three forage chicory cultivars that were developed in different parts of the world. Puna, 'INIA Le Lacerta' (Lacerta), and 'Forage Feast' were established on a Gilpin soil (fine-loamy, mixed, semiactive, mesic, Typic Hapludults) in replicated plots in 1997 and 1998, and herbage was used for chemical analyses and ruminant feeding assessments. Whitetail deer (*Odocoileus virginianus*), in a free-foraging situation, and sheep (*Ovis aries*), in two cafeteria trials, discriminated against Forage Feast. Deer selected Lacerta first; sheep did not exhibit a preference for Lacerta over Puna. Mature leaves from vegetative rosettes of the three cultivars had similar concentrations of crude protein, neutral detergent fiber, and acid detergent fiber ($P > 0.10$). In vitro organic matter disappearance and amino acid composition were also similar among the cultivars ($P > 0.05$). In all cultivars, approximately 65% of the total N occurred as protein amino acids. Nonprotein amino acids were not major constituents in any of the cultivars. Results suggest that differences in palatability and intake of chicory are related to the secondary plant metabolite composition of the herbage.

Since its release in 1985, Grasslands Puna chicory has been promoted extensively as a grazable forage for ruminants (Rumball, 1986; Barry, 1998). In New Zealand, where this forage cultivar was developed, thousands of hectares of Puna are established annually for finishing red deer (*Cervus elaphus*), sheep, and cattle (*Bos taurus*) (Moloney and Milne, 1993). Marketed for temperate, mediterranean, and tropical environments (Hare et al., 1987), Puna can now be found in pastures in Australia, North America, and South America, and it is being evaluated in Europe and Asia (Barry, 1998).

Forage chicory enhances pasture quality by improving seasonal distribution of high quality herbage (Kusmartono et al., 1996; Barry, 1998). New Zealand researchers found in vitro organic matter disappearance (IVOMD) of vegetative Puna chicory to be high (850 g kg^{-1}) and relatively constant throughout the growing season (Kusmartono et al., 1996), giving chicory a nutritional advantage over perennial ryegrass (*Lolium perenne* L.)–white clover (*Trifolium repens* L.) pasture during the summer–autumn period (Moloney and Milne, 1993; Niezen et al., 1993). Drought tolerance and high dry matter (DM) yields under summer conditions (Hare et al., 1987; Lancashire, 1978) ensure nutrient availability when livestock requirements are high (Hunt and Hay, 1990; Niezen et al., 1993; McCoy et al., 1997). Total N concentration in Puna is lower than in perennial ryegrass and red clover (*T. pratense* L.), but rumen N loss is less with chicory (Barry, 1998).

Livestock, including sheep (Fraser et al., 1988; Komolong et al., 1992), cattle (Nicol and Nicoll, 1987; Clark et al., 1990), and red deer (Niezen et al., 1993; Kusmartono et al., 1996) grazing Puna chicory in New Zealand achieved excellent rates of live-weight gain. Fraser et al. (1988) reported weight gains of 0.29 kg d^{-1} for lambs and 0.9 kg d^{-1} for calves grazing pure stands of chicory. Over a 6-wk period in late spring, lambs gained 0.27 kg d^{-1} (Komolong et al., 1992). Growth of lambs grazing chicory was 28% greater than that of lambs grazing ryegrass in the spring (Cruikshank, 1986) and 70% greater during the summer–autumn period (Barry, 1998). Voluntary feed intake was generally higher for animals grazing chicory (Barry, 1998).

Vigorous growth of Puna chicory and the apparent value of this cultivar as a pasture species in the northeastern (Reid et al., 1993; Jung et al., 1996) and midwestern (Volesky, 1996) USA led to evaluation of this cultivar in central Appalachia (Belesky et al., 1999; Turner et al., 1999). Growing lambs grazing chicory–orchardgrass (*Dactylis glomerata* L.) pastures refused to eat chicory even though the sward was maintained in a vegetative state (Belesky et al., 1996). These lambs, compared with ones grazing orchardgrass–white clover swards, had a lower average daily gain and a weight loss for the season. Lambs were observed nibbling leaves from flowering stalks after plants bolted (D. Belesky, personal communication, 1996).

Acceptance of or preference for a given herbage is a reflection of the chemical and physical characteristics of the plant material and the availability of other choices, all of which can be influenced by environmental factors (Church, 1979; Gershenzon, 1984). Puna chicory was developed under maritime conditions. A composite that is more densely leaved, more vigorous, and more uniform than the base population from which it was selected, Puna was the first commercial forage chicory

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Published in Agron. J. 94:1034–1042 (2002).

Abbreviations: ADF, acid detergent fiber; DM, dry matter; IVOMD, in vitro organic matter disappearance; NDF, neutral detergent fiber.

cultivar (Rumball, 1986; Hare et al., 1987). Other forage cultivars have subsequently become available in the USA. Lacerta is a synthetic variety derived from an ecotype grown by Uruguayan farmers. It is more uniform than the ecotype, but compared with Puna, it has a more erect growth habit and a lower proportion of plants remain vegetative in the first year (M. Rebuffo, personal communication, 1997). Forage Feast was derived from industrial chicory, a special group of root chicory varieties used for sugar production, and was selected for uniformity in vegetation and time of bolting (J. Kaye, personal communication, 1999).

This study was undertaken to compare the nutritive value and protein quality of these three forage cultivars when grown in the humid, temperate hill lands of central Appalachia. A second objective was to determine whether sheep will discriminate among the cultivars. This work is part of a series of investigations concerning the value of chicory in Appalachian hill-land pastures used for growing and finishing ruminants.

MATERIALS AND METHODS

Plot Establishment and Sample Collection

Experiments included three chicory cultivars that were developed for forage production. The New Zealand cultivar, Grasslands Puna, was obtained from Modern Forage Systems[1] (Ferndale, WA) and Cutting-Edge Agri Products (Lowry City, MO). Seeds for INIA Le Lacerta, which was developed in Uruguay, were obtained from Peterson Seed Company (now Independent Seeds, Savage, MN). Forage Feast chicory from France was obtained from Modern Forage Systems.

In 1997, replicated plots were established in a randomized complete block design on a Gilpin soil on a gently sloping upland site located in southern West Virginia. An existing pasture containing orchardgrass, tall fescue (*Festuca arundinacea*), white clover, and other species was killed with glyphosate [*N*-(phosphonomethyl) glycine] applied in midspring at a rate of 1.19 kg a.i. ha^{-1}. In 1997, a seedbed was prepared by rotary tilling the killed sod to a depth of 15 cm. Plots were sown with a mixture of chicory, 'Benchmark' orchardgrass (Southern States Coop., Richmond, VA), and 'Huia' white clover (Modern Forage Systems). Chicory seeds were mixed with sand and applied by hand to the plots at a rate of 5 kg ha^{-1}. Orchardgrass (18 kg ha^{-1} seed) and white clover (2 kg ha^{-1} seed) were then sown with a Brillion (Brillion, WI) seeder over all of the plots and alleyways. Plots were 3.7 by 12.2 m, separated by 0.9-m alleyways, and were replicated five times. Seeding was completed on 25 June, and no amendments were added during the establishment year.

By September 1997, the sward had developed into a vegetative canopy dominated by chicory. The plot area was surrounded by standard woven-wire fencing; however, the fencing was not effective against native whitetail deer. In early September, whitetail deer began to invade the plot area. Although the grazing was not planned, there were differences in the amount of herbage removed from individual plots. Grazing preference among the chicory cultivars was assessed on 12 Sept. 1997 using the point-quadrat method [25 contact points (every other quadrat in every other row) in a 1-m^2 area with 10-cm grid intervals] described by Warren-Wilson (1959). Assessments were made at least 1 m from plot edges and at three positions within each plot. The fraction of quadrats containing evidence of grazing was recorded at each position. Indications of grazing included partially eaten leaves or plant stubble but did not include simple trampling if there was no evidence of plant consumption. Herbage samples for chemical analyses were harvested on 17 Sept. 1997 and 19 Mar. 1998. Tops from 10 ungrazed chicory plants were collected randomly from each plot. Samples were frozen in liquid N, lyophilized, ground to pass a 1-mm screen using a Udy (Ft. Collins, CO) cyclone mill, and stored at −20°C until analyzed.

In 1998, new plots (4.3 by 12.2 m), replicated six times, were established near 1997 plots using the same plant species, seeding rates, and methods used in 1997. Alleyways within a replication were 0.9 m wide; alleyways between replications were 1.2 m wide. Seeding was completed on 19 May 1998. Fertilizer (33.6, 29.4, and 55.8 kg ha^{-1} N, P, and K, respectively) was applied on 21 May 1998 and again on 2 July 1998. By mid-July, canopies were 17 to 20 cm high and dominated by chicory. Forage samples were collected on 16 July 1998 using a sickle-bar mower set for a 5-cm cutting height. Herbage was gathered from a 1.8-m^2 harvest strip located in a relatively uniform, weed-free area of each plot at least 0.6 m from the plot edge. Individual chicory leaves (approximately 100 g fresh weight) were randomly selected from the harvest strip to create a cultivar sample for chemical analyses and processed for analytical procedures as described for 1997 samples. Remaining herbage in the harvest strip was weighed. Herbage from individual plots was then pooled by chicory cultivar, and three subsamples of each pool were collected for determination of botanical composition and DM content. The rest of the herbage was used in a cafeteria trial. Forage samples were collected again from previously uncut areas of the plots on 30 July 1998 and processed as described for forage collected on 16 July 1998.

In 1999, plots established in 1998 received a midspring application of fertilizer (30.5, 67.5, 112 kg ha^{-1} N, P, and K, respectively). Three of the replicates were designated for sampling for chemical analyses. On 10 May, each sample was a composite of rosettes from six randomly selected plants. By 23 June, chicory plants were rare in one of the replicates, and samples were not taken from those plots. Plants in the remaining plots had bolted, and leaf tissue at ground level was senescent. Tissue removed from the stems of six plants selected randomly within a plot was segregated into two samples: one contained only leaves, and the other was composed of buds and flowers. Samples collected in 1999 were processed as described for 1997 samples.

Cafeteria Trials

Twelve Dorset × Suffolk × Hampshire lambs (mean weight 39.5 kg) were used to determine sheep preferences for the chicory varieties. Before the trial, lambs were maintained on an orchardgrass–white clover pasture, received albendazole (Valbazen, Pfizer Animal Health, Exton, PA) drenches at 28-d intervals for parasite control, and had no previous exposure to chicory. Water and trace-mineralized salt were provided ad libitum. At least 24 h before the preference trial, lambs were removed from the pasture and confined in a barn with access to an attached 230-m^2 corral and free-choice orchardgrass hay and water. Herbage from chicory plots was weighed (0.45 kg) into 12-L plastic buckets (20 cm deep and 30 cm diam.; eight buckets per cultivar). Buckets were placed in groups (blocks) of three (one bucket of each cultivar) around the perimeter of the corral. Freshly cut herbage collected on 16 July was

[1] Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

offered to the lambs in midafternoon. The sheep showed little interest in any of the forage during the observation period but consumed or scattered the herbage overnight. Additional herbage, which had been refrigerated, was offered at 0900 h the following morning. All buckets were removed at 1000 h when it appeared that the sheep had eaten a significant portion of the offered forage. Refusals were collected, weighed, sealed in plastic bags, and refrigerated for assessment of botanical composition and DM content.

The preference trial was repeated with the same animals using forage harvested from chicory plots on 30 July. Herbage that had been refrigerated overnight was offered to sheep at 1020 h; buckets containing refusals were removed from the corral at 1055 h. Details of the procedures were identical to those described for the herbage harvested on 16 July.

Botanical compositions of offered and refused forage were determined within 48 h of collection. Three blocks (one sample of each cultivar per block) of refused forage were selected from each trial for manual separation into component species. Selection of blocks was based on the fresh weight of the refused forage, with the assumption that animal selection among the species components would be detected most readily in samples in which most, but not all, of the material had been consumed. Blocks in which all samples had fresh weights between 30 and 290 g were selected from the 17 July trial. The range was from 60 to 290 g for the 31 July trial. Separated plant materials and refusals not selected for separation were dried at 60°C in a forced-air oven and weighed. Component fractions of offered forage were recombined and ground to pass a 2-mm screen in a Wiley mill for forage quality analyses.

The relative amounts of chicory, orchardgrass, and all other species were expressed as a fraction of total DM in the sample. Fractional DM data were transformed using the arcsine function (Gomez and Gomez, 1984) before analysis using the general linear model (GLM) procedures of SAS (SAS Inst., 1990). Differences in the relative amount of chicory, orchardgrass, and all other species in relation to chicory cultivar and sample type (offered or refused) were initially assessed by separate analyses of variance in completely randomized design for each species (or species group) and trial. Confirmation of homogeneity of variance between the two trials for each species (Gomez and Gomez, 1984) permitted combining data across trials for final analyses. Chicory cultivar, date (trial), and interaction effects were assessed for each botanical component by analysis of variance. A second analysis-of-variance model was used to assess differences in the botanical composition of offered and refused forage (sample type) as well as chicory cultivar, trial, and associated interactions for each botanical component.

Chemical Analyses

Dried and ground plant samples were analyzed for DM and ash using AOAC (1990) procedures. For other determinations, subsamples were taken *as is*, and results were converted to a DM basis. Standard procedures for forage fiber analysis were used to determine neutral detergent fiber (NDF) (Goering and Van Soest, 1970; Van Soest et al., 1991) and acid detergent fiber (ADF) (Goering and Van Soest, 1970). The two-stage procedure of Tilley and Terry (1963), as described by Moore (1970), was used to determine IVOMD of herbage. Ruminal fluid used in the procedure was obtained from two ruminally cannulated steers offered alfalfa (*Medicago sativa* L.) and orchardgrass hay with supplemental chicory hay. Total N was determined simultaneously by combustion and gas chromatography techniques using a Carlo Erba Ea 1108 CHNS elemental analyzer (Fisons Instruments, Beverly, MA) (Pella and Colombo, 1978). Crude protein concentration was calculated by multiplying total N concentration (g kg^{-1} DM basis) by 6.25.

For amino acid analysis, duplicate subsamples (0.02 g) of chicory herbage were hydrolyzed in 6 *M* HCl as described by Nandula et al. (2000). Phenylthiocarbamyl derivatives of amino acids were prepared according to Cohen and Strydom (1988) and separated chromatographically using a Perkin Elmer (Norwalk, CT) Series 200 high-performance liquid chromatograph equipped with a Waters (Milford, MA) Pico-Tag Free Amino Acid Analysis column as described by Nandula et al. (2000). Eluent A contained 60 mL of acetonitrile and 940 mL of a buffer solution that was prepared by dissolving 19.0 g of sodium acetate trihydrate in 1 L of MilliQ water (Millipore, Bedford, MA) and then adding 0.5 mL of triethylamine and titrating the mixture to pH 6.40 with glacial acetic acid. Eluent B was prepared by mixing 600 mL of acetonitrile and 400 mL of MilliQ water. For quantification of S-containing amino acids, duplicate subsamples (0.02 g) were oxidized with performic acid (0.7 mL; Elkin and Griffith, 1985) before hydrolysis, following procedures of Spindler et al. (1984). The oxidation process was terminated by adding 0.1 mL of cold 9 *M* HBr. Oxidized samples were reduced to dryness in a Savant (Hicksville, IL) centrifugal vacuum evaporator, hydrolyzed, derivatized, and analyzed as described above. Data acquisition and peak quantification were accomplished using a PE Nelson (Norwalk, CT) Turbochrom 4 chromatographic data system. Amino acid N was determined as the sum of N contributions by individual amino acids quantified, calculated from residual molecular weights following hydrolysis. Non–amino acid N was calculated as the difference between total N and amino acid N.

Analysis-of-variance procedures were applied to the various sets of chemical data using the GLM procedures of SAS (SAS Inst., 1990). Nutritive value (ADF, NDF, IVOMD, and total N) of chicory was evaluated with a model that included main effects of date, cultivar, and the interaction. Amino acid composition of chicory components was evaluated with a model that included main effects of date, cultivar, tissue, and the interactions. When differences were detected among main effects and interactions, means were separated using least significant difference procedures (Snedecor and Cochran, 1980). For mean separations, tests of significance were made at the 0.05 level of probability.

RESULTS AND DISCUSSION

Early in September 1997, feral whitetail deer invaded the chicory plot area and provided an unplanned opportunity to evaluate ruminant discrimination among chicory cultivars. On 12 September, nearly all areas of Lacerta plots (91%) had been grazed to some extent. In contrast, 44% of the Puna plots and only 6% of the plots containing Forage Feast were grazed. The standard error of the mean for the five plots of each cultivar ranged from 0.01 for Forage Feast to 0.07 for Puna, indicating cultivar preference rather than random grazing. Deer continued to graze the plots after the assessment. Casual observation in early October indicated that all plots were grazed to a 5- to 10-cm stubble. Apparently, palatability of Puna and Forage Feast herbage improved, or the deer either acquired a taste for these two cultivars or consumed them when alternative forage was less desirable or less available.

Because chicory is a perennial and selective feeding behavior of whitetail deer was observed during the es-

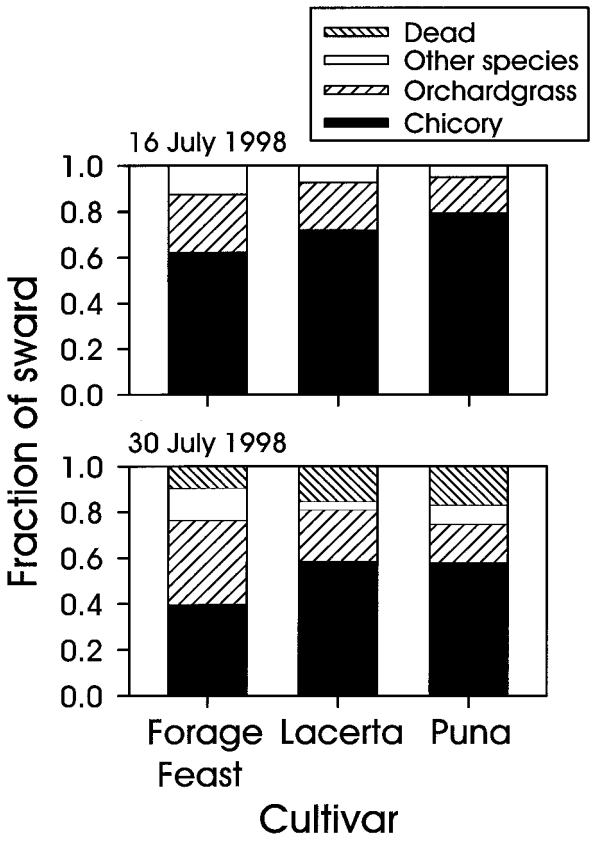

Fig. 1. Botanical composition of chicory plots harvested for 1998 cafeteria trials with sheep. Plots were sown to either Forage Feast, Lacerta, or Puna chicory in mixture with orchardgrass and white clover.

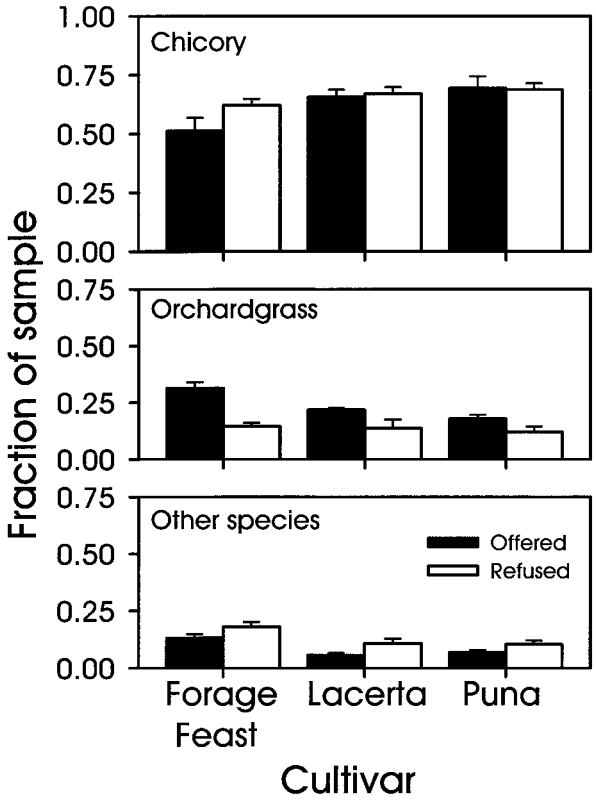

Fig. 2. Botanical composition of forage from chicory plots offered and refused in cafeteria trials with sheep. Values (dry matter basis) are means of two trials (n = 6). Error bars are the standard error of the mean.

tablishment year, new plots were established in 1998 to obtain herbage for cafeteria trails with sheep. Swards in plots established in May 1998 grew rapidly. By 16 July, chicory was the dominant component providing from 62 (Forage Feast) to 79% (Puna) of the DM (Fig. 1). Orchardgrass, the second largest contributor to DM, was most prevalent in Forage Feast plots (25%) and least prevalent in Puna plots (16%). White clover (averaging less than 1% of the DM), broadleaf weeds, and other grasses contributed the remaining DM. At the end of July, forage yield from the plots averaged 2000 kg ha^{-1}, with no significant differences among the cultivars ($P > 0.10$). Due to the density of the canopy, leaf senescence had begun to occur, and dead material, absent on 16 July, contributed up to 16% of the DM on 30 July (Fig. 1). The proportion of chicory in the sward decreased to an average of 52% on 30 July. A significant cultivar × trial interaction ($P < 0.05$) for orchardgrass reflected the increased contribution of orchardgrass in the Forage Feast plots between 16 and 30 July. There was no evidence of encroachment by whitetail deer from the time of chicory emergence to the end of July, perhaps because forage was readily available in surrounding fields and the plot area was visited fairly frequently by research personnel.

In two cafeteria trials conducted on 17 and 31 July 1998, sheep consumed an average of 64% of the DM offered in the forage mixtures. Comparisons of the botanical composition of offered and refused samples (Fig. 2) indicate that the sheep actively selected among plant species in the forage mixtures. Forage Feast herbage averaged 51 ± 5.5% (mean ± standard error) of the offered samples. In refused forage, Forage Feast herbage averaged 62 ± 2.7% of the sample. These values reflect consumption of other components in preference to Forage Feast. Sheep did not avoid either Puna or Lacerta as the chicory content of offered and refused samples differed by less than 1.5% for each cultivar. These cultivar differences resulted in a significant interaction ($P < 0.05$) between chicory cultivar and sample type (offered or refused samples) for chicory content (Table 1). The interaction between chicory cultivar and

Table 1. Analysis of variance and mean squares for species composition for 1998 cafeteria trials with sheep.†

		Plant species		
Source of variation	df	Chicory	Orchardgrass	Other
Cultivar (C)	2	173.7***	115.7***	205.7***
Sample type (S)‡	1	41.1 NS§	477.7***	159.2**
C × S	2	41.7*	43.6*	1.7 NS
Trial (T)	1	482.9***	28.5 NS	23.9 NS
C × T	2	19.0 NS	46.7*	29.9 NS
T × S	1	152.6**	220.6**	29.0 NS
C × T × S	2	2.6 NS	3.1 NS	23.2 NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Data from two cafeteria trials were combined and arcsine transformed prior to analysis.
‡ Offered or refused samples.
§ NS, not significant at the 0.10 probability level.

Table 2. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and in vitro organic matter disappearance (IVOMD) [dry matter (DM) basis] of herbage from chicory cultivars offered in cafeteria trials with sheep in July 1998.†

	Puna		Lacerta		Forage Feast		Source of variation			
Constituent	16 July	30 July	16 July	30 July	16 July	30 July	SE	Cultivar (C)	Date (D)	C × D
	g kg^{-1} DM									
NDF	343	388	374	404	380	457	0.58	***	***	**
ADF	279	277	302	262	240	293	0.87	NS‡	NS	***
IVOMD	710	604	715	629	676	532	1.63	**	***	NS

** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Herbage samples also contained orchardgrass and small quantities of other plant species.
‡ NS, not significant ($P > 0.01$).

trial date was not significant ($P > 0.10$) for chicory content, indicating that exposure of the sheep to chicory during the first trial did not result in an acquired taste that influenced the response during the second trial. Orchardgrass was preferred to other plant species as its content was consistently lower in the refused samples than in the offered samples (Fig. 2). The significant interaction ($P < 0.05$) between cultivar and sample type for orchardgrass was due to an increased preference for orchardgrass when in a mixture with Forage Feast (Table 1 and Fig. 2). The proportion of other species was consistently higher in the refused samples than in the offered samples. This observation is not surprising because unpalatable horse nettle (*Solanum carolinense* L.) was a common constituent.

Plant selection by herbivores can be influenced by the physical and chemical characteristics of the herbage and the impact of environmental factors such as climate, soil type and fertility, and topography on these characteristics (Church, 1979). Chicory cultivars assessed in this study were established at the same site with the same companion species, and there were no obvious physical characteristics of leaves that might account for observed animal feeding behaviors. The cultivars have distinctly different origins, may have inherent differences in chemical composition, and could differ in their metabolic responses to the same growing conditions. Discrimination among the cultivars by both whitetail deer and sheep suggests that cultivar selection has a chemical basis.

High fiber and low crude protein are associated with low preference (Church, 1979). Analyses of chicory rosettes harvested within 1 wk of the 1997 grazing assessment revealed similar levels of NDF (287–305 g kg^{-1}), ADF (220–223 g kg^{-1}), and IVOMD (786–803 g kg^{-1}) among the three cultivars ($P > 0.10$). Cultivar selection was therefore not directly attributable to differences in fiber content or digestibility of the chicory herbage. In herbage used in cafeteria trials, the NDF concentration ranged from 343 (Puna) to 380 g kg^{-1} (Forage Feast) on 16 July and from 388 (Puna) to 457 g kg^{-1} (Forage Feast) on 30 July (Table 2). The interaction between plot and harvest date was significant for both NDF ($P < 0.01$) and ADF ($P < 0.001$), reflecting the differential contribution of orchardgrass to the botanical composition of chicory plots. The significant ($P < 0.001$) cultivar effect is due to the different proportions of chicory and orchardgrass in the herbage mixtures (Fig. 1). The ADF concentration in offered herbage did not vary ($P > 0.10$) with plot or harvest date (Table 2). Plots containing Forage Feast had lower IVOMD ($P < 0.01$) than did plots containing Lacerta and Puna. The IVOMD for all plots was lower ($P < 0.001$) at the end of July than in the middle of the month, indicative of physiological maturation of the plants between the two harvest dates. Fiber and digestibility analyses were not conducted on pure chicory samples. Because of similarities observed in fiber and digestibility of 1997 samples, pure chicory samples were reserved for investigation of other potential chemical differences among cultivars.

Total N concentration in pure chicory samples collected from plots harvested for 1998 cafeteria trials ranged from 24.5 (Lacerta) to 25.9 g kg^{-1} (Forage Feast) on 16 July. On 30 July, total N concentrations ranged from 20.5 (Lacerta) to 21.9 g kg^{-1} (Forage Feast). At each harvest date, total N concentration was similar ($P > 0.10$) among the cultivars, and crude protein averaged 157 g kg^{-1} on 16 July and 133 g kg^{-1} on 30 July. The decrease ($P < 0.001$) in crude protein concentration between the two harvest dates is consistent with aging of the plant material (Lyttleton, 1973).

The amino acid composition of chicory herbage collected on 17 Sept. 1997 is given in Table 3. Concentrations of individual amino acids were similar ($P > 0.10$) among the three cultivars, indicating consistency in pro-

Table 3. Amino acid concentration in intact chicory rosettes harvested 17 Sept. 1997 from cultivars established 25 June 1997 and grazed by whitetail deer in September 1997.

	Cultivar		
Amino acid†	Puna	Lacerta	Forage Feast
	μmol g^{-1} DM		
Cys	8.8‡	9.5	8.9
Asx	74.0	70.4	76.5
Glx	83.0	79.8	86.1
Hyp	1.9	1.9	2.1
Ser	41.0	38.4	42.5
Gly	73.3	69.3	77.6
His	12.7	12.3	13.6
Arg	40.2	36.9	41.2
Thr	40.5	37.9	42.8
Ala	72.9	67.9	76.4
Pro	46.0	42.3	48.0
Met	12.6	11.8	13.3
Tyr	18.9	16.6	18.8
Val	56.3	52.5	59.4
Ile	41.6	39.3	44.1
Leu	72.5	67.6	77.0
Phe	36.7	33.9	38.8
Lys	39.3	37.3	40.7

† Asx, aspartate plus asparagine; Glx, glutamate plus glutamine; Hyp, hydroxyproline.
‡ Value are means of five field replications. Means within rows are not significantly different ($P \geq 0.05$).

Table 4. Amino acid concentration in rosette leaves from chicory cultivars established 19 May 1998 and used for cafeteria trials with sheep in July 1998.

	Cultivar						Significance		
	16 July 1998			30 July 1998					
Amino acid†	Puna	Lacerta	Forage Feast	Puna	Lacerta	Forage Feast	Cultivar	Date	Cultivar × date
	μmol g^{-1} DM								
Cys	9.0‡	9.7	9.8	9.1	8.6	8.7	NS§	*	NS
Asx	83.5	82.3	82.7	66.3	60.9	69.2	NS	***	NS
Glx	95.9	95.8	96.2	75.2	69.5	80.5	NS	***	NS
Hyp	1.6	1.6	1.6	1.5	1.5	1.5	NS	NS	NS
Ser	47.1	45.1	46.9	35.9	33.4	37.1	NS	***	NS
Gly	91.7	87.1	90.7	72.2	66.3	77.3	NS	***	NS
His	16.7	16.3	16.9	13.8	12.4	14.2	NS	***	NS
Arg	52.1	49.7	50.2	40.2	35.3	43.4	NS	***	NS
Thr	47.7	47.1	47.5	41.0	37.4	42.5	NS	***	NS
Ala	84.7	80.3	86.0	66.5	61.6	70.0	NS	***	NS
Pro	53.5	50.1	53.0	43.2	39.1	47.5	*	***	NS
Met	14.0	13.4	14.2	11.8	11.4	12.0	NS	***	NS
Tyr	24.0	22.8	23.6	19.6	18.2	20.7	NS	***	NS
Val	67.7	62.7	67.2	55.7	51.6	60.7	NS	***	NS
Ile	49.6	45.3	50.1	38.5	36.1	39.7	NS	***	NS
Leu	87.9	79.9	93.2	70.6	64.0	72.9	*	***	NS
Phe	44.5	41.2	45.6	34.4	32.7	38.0	NS	***	NS
Lys	53.8	48.8	55.6	41.5	40.2	44.0	NS	***	NS

* Significant at the 0.05 probability level.
*** Significant at the 0.001 probability level.
† Asx, aspartate plus asparagine; Glx, glutamate plus glutamine; Hyp, hydroxyproline.
‡ Values are means of six field replications.
§ NS, not significant ($P > 0.10$).

tein quality. The same was true of amino acids in herbage collected on 16 July 1998 (Table 4). On 30 July 1998, only proline concentrations differed among the cultivars ($P < 0.05$), with Forage Feast having the highest concentration and Lacerta having the lowest concentration of this amino acid (Table 4). Amino acid concentrations in 1997 samples (Table 3) generally fell within the range defined by samples collected in July 1998 (Table 4). In leaves from young, actively growing plants, as much as 50% of the total soluble protein is the CO_2–fixing enzyme ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39) (Lyttleton, 1973); thus, similarities in amino acid composition among the cultivars (Tables 3 and 4) are not unexpected. The decrease [$P < 0.001$, except for cysteine ($P < 0.05$) and hydroxyproline ($P > 0.10$)] in concentration of amino acids between 16 July and 30 July (Table 4) corresponds with the decrease in total N concentration. Nonprotein amino acids, which can adversely affect livestock, were not evident in leaves of any of the cultivars.

Table 5. Amino acid concentration in chicory rosettes harvested on 19 Mar. 1998 from cultivars established 25 June 1997 and grazed by whitetail deer in September 1997.

	Cultivar			
Amino acid†	Puna	Lacerta	Forage Feast	Significance
	μmol g^{-1} DM			
Cys	18.7ab‡	20.3a	17.4b	*
Asx	164.4a	167.3a	133.3b	**
Glx	204.8ab	211.7a	164.2b	**
Hyp	4.3	4.6	4.2	NS§
Ser	67.8ab	75.2a	60.6b	**
Gly	135.1ab	141.5a	118.8b	*
His	29.7a	27.6a	24.2b	*
Arg	66.3a	67.2a	53.6b	*
Thr	63.7ab	68.3a	55.2b	***
Ala	129.8a	131.7a	111.7b	*
Pro	87.1a	80.1ab	72.6b	*
Met	23.3ab	25.0a	21.2b	*
Tyr	33.0a	34.4a	28.4b	*
Val	100.4a	103.5a	87.9b	*
Ile	77.2a	78.5a	67.5b	*
Leu	128.9a	132.7a	112.5b	*
Phe	61.5a	61.6a	52.7b	*
Lys	90.6a	89.1a	77.9b	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Asx, aspartate plus asparagine; Glx, glutamate plus glutamine; Hyp, hydroxyproline.
‡ Values are means of five field replications. Within rows, means followed by the same letter are not significantly different at the probability level indicated in the last column.
§ NS, not significant ($P \geq 0.05$)

Concentrations of amino acids in chicory rosettes collected on 19 Mar. 1998 from plots grazed by deer in 1997 (Table 5) were approximately twice ($P < 0.001$) those in tissues collected the previous fall (Table 3). Cultivars also varied in amino acid composition in early spring, with Forage Feast tending to have lower amino acid concentrations than Lacerta and Puna. Springtime mobilization of amino acids from chicory roots (Fouldrin and Limami, 1993) is probably responsible for these higher concentrations. Clapham et al. (2001) reported uniformity in developmental dynamics in Puna chicory, and the timing of amino acid mobilization could be characteristic of a cultivar. Data in Table 5 suggest that mobilization in Forage Feast either precedes or lags behind that in the other two cultivars. In leaves collected later in the spring in 1999 from plants established in 1998, concentrations of individual amino acids were similar ($P > 0.10$) among the cultivars at the same physiological stage (Table 6). Values for leaves of vegetative rosettes were reminiscent of those for rosettes harvested during the establishment year (Table 4). Amino acid concentrations in leaves from stems of bolting plants were lower ($P < 0.05$) than those from vegetative rosettes (Table 6).

Table 6. Amino acid concentration in chicory leaves and floral material collected in 1999 from cultivars established 19 May 1998.

	Cultivar									Significance		
	10 May rosette leaves			23 June stem leaves			23 June buds and flowers					
Amino acid†	Puna	Lacerta	Forage Feast	Puna	Lacerta	Forage Feast	Puna	Lacerta	Forage Feast	Cultivar	Tissue	Cultivar × tissue
	μmol g^{-1} DM											
Cys	12.0‡	11.3	10.4	7.7	9.2	8.9	13.3	13.8	14.2	NS§	***	NS
Asx	77.1	64.0	65.7	48.3	52.0	52.7	68.8	69.5	74.3	NS	***	NS
Glx	91.6	76.5	78.4	55.4	62.3	59.8	75.9	75.6	81.4	NS	***	NS
Hyp	2.2c	1.7c	2.1c	1.7c	1.7c	1.9c	10.4a	7.5b	8.2b	**	***	*
Ser	45.3	38.8	40.1	29.4	33.8	32.5	43.5	44.1	45.3	NS	***	NS
Gly	80.3	69.7	69.8	55.3	63.9	59.6	63.7	64.0	67.5	NS	**	NS
His	14.4ab	12.1bcd	11.9cd	9.0e	11.6cde	11.2de	14.4abc	14.0abcd	16.3a	NS	***	*
Arg	35.5	30.7	31.3	23.3	27.2	25.6	28.3	30.2	30.6	NS	***	NS
Thr	42.6	37.0	38.1	29.4	34.1	33.0	35.6	38.0	37.4	NS	**	NS
Ala	77.5	67.2	67.9	54.2	62.8	58.8	63.3	64.0	65.5	NS	*	NS
Pro	48.9c	43.2d	44.6cd	36.7e	41.0de	40.7de	66.3b	60.8b	77.5a	*	***	**
Met	13.9	12.9	11.9	10.1	11.9	11.1	13.2	13.5	13.3	NS	**	NS
Tyr	23.7a	19.0b	20.3ab	15.3c	19.8b	19.3bc	17.8bc	19.2bc	20.5ab	NS	*	*
Val	60.4	52.3	52.4	42.3	48.6	45.1	49.3	50.1	51.1	NS	**	NS
Ile	44.8	38.5	39.1	31.6	36.4	33.8	38.3	38.6	40.0	NS	**	NS
Leu	80.8	69.4	69.9	56.6	65.3	61.0	62.2	63.9	64.5	NS	**	NS
Phe	47.3	40.9	41.0	30.6	37.7	35.0	32.5	35.6	32.8	NS	**	NS
Lys	48.0	41.0	41.7	31.3	36.5	34.8	44.3	45.0	46.2	NS	***	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Asx, aspartate plus asparagine; Glx, glutamate plus glutamine; Hyp, hydroxyproline.
‡ Mean values are given for three replicates on 10 May and two replicates on 23 June. Duplicate analyses were performed for each replicate. When a significant cultivar × tissue interaction effect was detected, means were separated using least significant difference procedures. Within rows, means followed by the same letter are not significantly different ($P \geq 0.05$).
§ NS, not significant ($P > 0.10$).

Translocation of metabolic resources to reproductive sinks may account for the difference in amino acid concentration between leaf types. Consumption of leaves from bolting Puna stems by sheep that avoided vegetative rosettes (D. Belesky, personal communication, 1996) is apparently not linked to general protein concentration of the respective tissues. Plants selected by grazing livestock have been reported to change diurnally (Kirby and Stuth, 1982) and seasonally (Funk et al., 1987). In general, selectivity by livestock for highly digestible plant parts is especially evident during summer when overall forage quality and availability are low. Hakkila et al. (1987) reported that the diet of steers grazing range grasslands changed with advancing season to maximize dietary quality. In chicory flowers and buds (Table 6), concentrations of cysteine, hydroxyproline, and proline exceeded those in leaf tissues ($P < 0.001$). Concentrations of other amino acids in flowers and buds were within the range observed for leaf tissues. Significant cultivar × tissue interaction effects for hydroxyproline, histidine, proline, and tyrosine reflect differences in the distribution of amino acids in foliar and reproductive tissues by the three cultivars.

Table 7. Amino acid N in leaf tissue collected on 16 July 1998 from forage chicory cultivars established 19 May 1998.

	N concentration†		
Amino acid‡	Puna	Lacerta	Forage Feast
	μg^{-1} DM		
Cys	129	136	137
Asx	1 170	1 153	1 159
Glx	1 344	1 342	1 348
Hyp	22	22	22
Ser	660	632	657
Gly	1 285	1 220	1 271
His	702	685	710
Arg	2 920	2 785	2 813
Thr	668	660	665
Ala	1 187	1 125	1 205
Pro	750	702	747
Met	196	188	199
Tyr	336	319	331
Val	948	878	941
Ile	695	635	702
Leu	1 231	1 119	1 306
Phe	623	577	639
Lys	1 507	1 367	1 558
sum	16 373	15 547	16 410

† Amino acid N was calculated using data from Table 4 and residual molecular weights for amino acids following hydrolysis. Values are means of six field replications. Means within rows are not significantly different ($P \geq 0.05$).
‡ Asx, aspartate plus asparagine; Glx, glutamate plus glutamine; Hyp, hydroxyproline.

The amount of N contributed by each of the individual amino acids in chicory samples collected on 16 July 1998 is presented in Table 7. These values, calculated from amino acid concentration data given in Table 4, represent contributions by both protein-derived and free amino acids. Tryptophan, which is destroyed by the hydrolytic procedures used, is not represented. If the low tryptophan concentrations reported in other plant materials [45–96 mg g^{-1} sample N (DM basis); Sosulski and Imafidon, 1990] are also characteristic of chicory, then total amino acid N accounts for 63 (Lacerta and Forage Feast) to 66% (Puna) of the N in each cultivar. The remainder represents nonprotein nitrogenous constituents. A number of plants have N-containing metabolites that adversely impact forage utilization by livestock (Hoveland and Monson, 1980). Similarities in nonprotein N concentrations among the chicory cultivars suggest that avoidance of Forage Feast by whitetail deer and sheep is

Table 8. Mean monthly temperature and monthly precipitation for 1997, 1998, and 1999, and 30-yr mean values for each parameter at Beckley, WV (37°45′ N; 81°15′ W; 850 m above sea level).

Month	Temperature				Precipitation			
	30-yr mean	1997	1998	1999	30-yr mean	1997	1998	1999
	°C				mm			
Jan.	−1.6	−0.8	2.3	2.2	74	66	122	116
Feb.	0.1	3.8	2.7	2.0	75	52	126	69
Mar.	5.6	7.3	4.9	2.3	86	163	96	78
Apr.	10.7	8.3	10.8	12.5	87	67	120	91
May	15.3	12.3	16.6	15.9	101	108	193	35
June	19.0	18.6	19.2	20.3	98	119	178	32
July	20.9	21.5	21.0	23.1	119	110	114	96
Aug.	20.4	19.3	21.1	20.2	86	83	39	90
Sept.	17.1	16.1	19.4	16.7	85	37	48	114
Oct.	11.3	10.8	12.1	11.1	73	23	39	55
Nov.	6.3	3.3	6.5	8.4	76	75	67	63
Dec.	1.1	0.5	2.9	2.2	82	56	110	47

directed by some other chemical factor. However, the distribution of N among antiquality constituents such as NO_3 and alkaloids in each cultivar needs to be investigated. Belesky et al. (2000) reported higher NO_3 concentrations in Puna herbage during a dry year.

Coley et al. (1985) proposed that the nature and quantity of plant constituents that impact herbivory are determined by the resources available and conditions encountered in the local habitat. Observed feeding behaviors could reflect differential impacts of the Appalachian environment on the individual cultivars. Plants experienced unseasonable weather conditions during 2 of the 3 yr of this study (Table 8). Temperatures and precipitation in June, July, and August of 1997 were close to the respective 30-yr means for the area. In 1998 and 1999, temperatures during the growing season were also similar to the 30-yr norm. Precipitation is typically well distributed throughout the growing season. In 1998, precipitation during April, May, and June was nearly twice that normally received in the area. From August through October, the area received approximately half of the normal monthly amounts of rainfall, but the annual precipitation (125 cm) was still 20% above normal (104.2 cm). In 1999, May, June, and July were exceptionally dry, and precipitation for the year (88.6 cm) was 15% below normal. These fluctuations in local conditions did not result in cultivar differences in fiber or crude protein concentrations or protein quality (Tables 3, 4, and 6). Gianquinto and Pimpini (1989) noted that chicory is sensitive to fluctuations in soil temperature, and farmers located on the North Island of New Zealand noted physiological responses to the cool summer and autumn of 1992 (Moloney and Milne, 1993). Soil temperature effects on the chemical composition of chicory herbage have not been reported. Disparate day and night temperatures, combined with high elevation and varying slope aspects characteristic of Appalachian hill lands, may promote production of chemicals that discourage herbage utilization by ruminants.

Puna, Lacerta, and Forage Feast, grown under climatic and edaphic conditions typical of the central Appalachian Plateau, were equivalent in nutritive value. Fiber and protein components of forage quality are correlated with voluntary consumption but are related to rate of digestion, rather than sensory perception, of the herbage (Church, 1979). Secondary metabolites are often responsible for diminished palatability of forage (Rosenthal and Janzen, 1979), and the occurrence and concentration of secondary compounds in a plant are determined by genetic factors and influenced by environmental conditions (Tribe and Gordon, 1950). A number of compounds, including sesquiterpene lactones, tannins, and other phenolic compounds, have been reported to occur in chicory (Rees and Harborne, 1985; Barry, 1998). Studies are underway to determine whether variations in concentrations of any of these constituents occur among Lacerta, Puna, and Forage Feast. Samples collected during the course of this study offer an opportunity to interpret analytical results in terms of animal responses. Knowledge of specific compounds that adversely impact forage acceptability is fundamental to the development of strategies for using chicory to enhance pasture quality.

ACKNOWLEDGMENTS

Robert C. Arnold, Kenneth N. Harless, Joyce M. Ruckle, and Nathan Wade Snyder provided technical assistance during some phases of this research. This help and suggestions offered by Dr. David P. Belesky are gratefully acknowledged.

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